PATENT SPECIFICATION (11) 1 200 582

1200582

NO DRAWINGS

(21) Application No. 3292/69 (22) Filed 21 Jan. 1969
(31) Convention Application No. 715 149 (32) Filed 22 March 1968 in
(33) United States of America (US)
(45) Complete Specification published 29 July 1970
(51) International Classification A 23 k 1/18
(52) Index at acceptance
A2B J3F2 J3G1

(54) DOG FOOD COMPOSITIONS

ERRATA

SPECIFICATION No. 1,200,582

Page 1, line 67, *for* "ratios." *read* "rations."
Page 2, line 104, *for* "ribonucleotides" *read* "ribonucleosides"
Page 4, Table 1, Column 4, 3rd line down, *for* "523.1(15)" *read* "53.1(15)"
Page 6, Table 3, 1st line, *for* "Ratio" *read* "Ration"
Page 11, Example 9, Heading, 3rd line, *for* "ratio" *read* "ration"
Page 14, line 69, *for* "5300" *read* "5400"
Page 14, lines 70 to 72, *should read*, "ml. One sample of the hydrolyzate (800 ml.) was diluted by the addition of water (3200 ml.) and marked as supplement A. A second"

THE PATENT OFFICE
17th May 1971

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On the other hand, it is generally recognised that 2'- and 3'-nucleotides, although isomers of the 5'-nucleotides, possess very little if any flavour enhancing properties. It has been stated, in the prior art, that purine and pyrimidine bases, their nucleosides and their 2'- and 3'-nucleotides have little flavour, whilst the 5'-nucleotides have a very agreeable taste. It has therefore been the practice, for this purpose, to try to prepare 5'-nucleotides by methods which avoid the formation of 2'- and 3'- nucleotides; and the chemical degradation or hydrolysis of ribonucleic acid materials has been avoided, since the products of such processes contain 2'- and 3'-nucleotides, but not 5'-nucleotides.

However, in contra distinction to the effect observed in humans, it has been found that the addition of 5'-nucleotides to dog foods produces little or no enhancement of the flavour or acceptability of such foods when comprising a dog food and a flavour enhancer selected from the following: ribonucleosides, 2',3'-ribonucleotides, poly - 2',3' - ribonucleotides, purine, pyrimidine, ribonucleic acid, chemically hydrolysed ribonucleic acid-containing materials, thymidine, xanthosine, hypoxanthine, xanthine, uric acid, cytosine, 5-methyl-cytosine, uracil, thymine, orotic acid, adenine, guanine, ureidosuccinic acid, dihydro-DL-orotic acid, dihydrouracil, dihydrothymine, dihydro-6-methyl-uracil, allantoin and salts and mixtures thereof.

The expression "2',3'-ribonucleotides", as used herein, should be understood to include 2'-ribonucleotides, 3'-ribonucleotides and 2',3'-ribonucleotides, as well as salts and mixtures thereof; and the expression "poly-2',3'-ribonucleotides" should be understood to include polyribonucleotides in which the polymer chain terminates in a 2'-ribonucleotide, a 3'-ribonucleotide or a 2',3'-ribonucleotide unit, as well as salts and mixtures thereof.

Examples of the 2',3'-ribonucleotides which

[*Price 5s. 0d. (25p)*]

SEE ERRATA SLIP ATTACHED

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(54) DOG FOOD COMPOSITIONS

(71) We, RALSTON PURINA COMPANY, of 835 South Eighth Street, St. Louis, Missouri 63199, United States of America, a Corporattion organised and existing under the Laws of the State of Missouri, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to dog food compositions containing adducts which enhance the flavour and acceptability of the compositions.

It is well known that 5'-nucleotides and 5'-ribonucleotides are useful for the flavour enhancement of human foods. 5'-Nucleotides, such as 5'-guanylic acid and 5'-inosinic acid and their salts, are potent flavour enhancers which may be incorporated into foods for human consumption. Certain 5'-nucleotides, such as sodium 5'-inosinate and sodium 5'-guanylate, in combination with monosodium glutamate, have also been found to enhance the magnitude of the chorda tympani response in rats to stimulation of the tongue.

On the other hand, it is generally recognised that 2'- and 3'-nucleotides, although isomers of the 5'-nucleotides, possess very little if any flavour enhancing properties. It has been stated, in the prior art, that purine and pyrimidine bases, their nucleosides and their 2'- and 3'-nucleotides have little flavour, whilst the 5'-nucleotides have a very agreeable taste. It has therefore been the practice, for this purpose, to try to prepare 5'-nucleotides by methods which avoid the formation of 2'- and 3'- nucleotides; and the chemical degradation or hydrolysis of ribonucleic acid materials has been avoided, since the products of such processes contain 2'- and 3'-nucleotides, but not 5'-nucleotides.

However, in contra distinction to the effect observed in humans, it has been found that the addition of 5'-nucleotides to dog foods produces little or no enhancement of the flavour or acceptability of such foods when fed to dogs. It has therefore not been possible to enhance the flavour and acceptability of dog foods by a method parallel to the incorporation of 5'-nucleotides in foods for human consumption.

It has now surprisingly been found that 2'-, 3'-nucleotides, poly 2',3'-nucleotides, nucleosides and certain other related compounds, are effective in imparting a significantly enhanced flavour or palatability to dog foods. This finding, which is unexpected in view of the prior art, is apparently attributable to a biological specificity of such materials in dogs, which differs from that observed in humans, as regards the property of flavour enhancement. Whilst the underlying mechanism is not fully understood, it has been shown that different species of dogs show a positive and definite preference for foods incorporating such materials over control ratios. Accordingly, the invention provides dog food compositions which have an enhanced flavour and acceptability when fed to dogs.

The invention consists in a composition comprising a dog food and a flavour enhancer selected from the following: ribonucleosides, 2',3'-ribonucleotides, poly - 2',3' - ribonucleotides, purine, pyrimidine, ribonucleic acid, chemically hydrolysed ribonucleic acid-containing materials, thymidine, xanthosine, hypoxanthine, xanthine, uric acid, cytosine, 5-methyl-cytosine, uracil, thymine, orotic acid, adenine, guanine, ureidosuccinic acid, dihydro-DL-orotic acid, dihydrouracil, dihydrothymine, dihydro-6-methyl-uracil, allantoin and salts and mixtures thereof.

The expression "2',3'-ribonucleotides", as used herein, should be understood to include 2'-ribonucleotides, 3'-ribonucleotides and 2',3'-ribonucleotides, as well as salts and mixtures thereof; and the expression "poly-2',3'-ribonucleotides" should be understood to include polyribonucleotides in which the polymer chain terminates in a 2'-ribonucleotide, a 3'-ribonucleotide or a 2',3'-ribonucleotide unit, as well as salts and mixtures thereof.

Examples of the 2',3'-ribonucleotides which

[Price 5s. 0d. (25p)]

SEE ERRATA SLIP ATTACHED

may be used in the invention include purine-based materials, such as 3'-adenylic acid, 3'-guanylic acid, 2',3'-adenylic acid, 2',3'-inosinic acid, 2'-xanthylic acid; and pyrimidine-based materials, such as 2',3' cytidylic acid and 2',3'-uridylic acid. Other 2',3'-ribonucleotide compounds having a free phosphate or a phosphate salt radical in the 2', 3'-, or 2' and 3' positions of the ribose portion of the molecule are well known.

The separation of 2'-ribonucleotides from 3'-ribonucleotides, and of 2'- and 3'-ribonucleotides from 2',3'-ribonucleotides, and the separation of poly-2',3'-ribonucleotides from each other, can all present difficulties. It is therefore often convenient to use a mixture of such materials, in pure or impure form, in the present invention. In particular, mixtures of 2'-, 3'-, and 2',3'-ribonucleotides can be prepared by the chemical hydrolysis of ribonucleic acid-containing materials, such as yeasts, and the resulting hydrolysates containing such mixtures can be directly utilised in the invention. The expression " ribonucleic acid-containing materials ", as used herein, should be understood to mean polymeric compounds or materials found in living tissues which include monomeric units consisting of purine or pyrimidine bases combined with ribose units. In the case of nucleotides and polynucleotides, the units include phosphate groups at the 2'-, 3'-, or 2'- and 3'-carbon positions, whereas the nucleosides are phosphate-free. Useful ribonucleic acid-containing materials include such natural sources as yeast (e.g. dried brewer's yeast or torula yeast) and animal tissues. It is known that the chemical hydrolysis of such materials with aqueous alkali produces a hydrolysate containing a mixture of 2'-, 3'- and/or 2',3'-ribonucleotides, and/or poly-2',3'-ribonucleotides and/or ribonucleosides—the exact composition of the hydrolysate being dependent upon the extent of hydrolysis (i.e. partial or complete), the conditions of hydrolysis and the nature of the starting material.

In the past, it has been conventional to carry out the hydrolysis of ribonucleic acid-containing materials at a pH below 12, for example using 1.5 to 2.0 milli-equivalents of alkali per gram of yeast starting material. The hydrolysis has been carried out at room or elevated temperatures, for a sufficient length of time to give the desired degree of hydrolysis. Such conventional methods of hydrolysis may be used to produce hydrolysates which can be incorporated as the flavour enhancers in the compositions of the present invention. However, such conventional alkaline hydrolysis has been found to contribute to an undesirable growth of certain thermophilic bacteria. It has now been found that a relatively bacteria-free hydrolysate, useful in the compositions of the present invention, may be produced by carrying out the hydrolysis at a pH of at least 12.

In our copending application No. 35494/69 (Serial No. 1,200,583) there is described and claimed a process for the hydrolysis of a ribonucleic acid-containing material, in which said hydrolysis is conducted in an aqueous medium, at a pH of at least 12, for a period of at least three hours.

The pH of the hydrolysis mixture must be maintained at 12 or over during the process and, since the pH normally decreases slightly (e.g. by 0.2 to 0.4) during the course of hydrolysis, it is preferred to operate at a pH of at least 12.5 or 13. For example, using yeast as the ribonucleic acid-containing material, approximately 3.5 milli-equivalents of an alkaline compound, such as potassium hydroxide, per gram of yeast is found to give a suitable pH. The hydrolysis may be carried out at room temperatures (i.e. 15°C to 25°C) or at elevated temperatures. Preferably, the hydrolysis is carried out at a temperature between 25°C and 55°C. Also, it is preferred to continue the hydrolysis for a period from 12 to 24 hours. Any conventional alkaline compound may be used, such as sodium hydroxide or potassium hydroxide.

The hydrolysates resulting from the hydrolysis of ribonucleic acid-containing materials are in the form of aqueous slurries or suspensions; and they may be used directly for making the compositions of the invention by addition to the dog food, for example by spraying. If desired, the hydrolysates may be neutralized before they are added to the dog food, by the addition of an acid, such as acetic acid, phosphoric acid or hydrochloric acid.

When ribonucleotides are used as the flavour enhancers in the compositions of the invention, suitable examples include adenosine, guanosine, cytidine and uridine, as well as other purine or pyrimidine bases combined with ribose.

When the flavour enhancers used in the compositions of the invention are the salts of the above listed compounds or materials, it is generally preferred to use the alkali metal salts, such as the sodium or potassium salts, or the alkaline earth metal salts, such as the calcium salts. The selection of the particular salt or salts to be used is often governed by the composition of the dog food to which the flavour-enhancing compounds or materials are to be added, and by the nutritional requirements of the dogs being fed.

It has been found that the addition of only small amounts of the above flavour enhancers can have a significant effect on the dog food. In general, the use of as little as four parts per million of the flavour enhancer, on the basis of the dog food, is found to produce a definite enhancement of

the flavour and acceptability of the food. Generally, it is preferred to use from 8 to 80 parts per million of the flavour enhancer, on the basis of the dog food, in the compositions of the invention. When using a hydrolysate, as described above, as the flavour-enhancing material, it is preferred to use from 0.2 to 2.0 grams (dry weight) of the material per pound of dog food; and, most preferably, about one gram of the hydrolysate per pound of dog food: this is equivalent to approximately 80—100 parts per million parts of dog food of the active ribonucleoside, 2',3'-ribonucleotide, polyribonucleotide and poly-2',3'-ribonucleotide flavour enhancers contained in the hydrolysate. It will be understood that the hydrolysates described above contain a mixture of such polymeric and monomeric materials and salts thereof which, in admixture, produce a distinct flavour-enhancing effect.

The flavour, acceptability and/or palatability of the compositions of the invention may further be enhanced by the addition of a salt of glutamic acid. Monosodium glutamate is the preferred salt for this purpose, but other salts, such as the potassium and calcium salts, may also be used. It is generally preferred to use at least twenty parts per million of the glutamic acid salt, on the basis of the dog food.

The compositions of the invention may additionally contain small amounts of stabilising agents, so as to stabilise the compositions against deterioration of the enhanced flavour during storage over extended periods of time. Useful stabilising agents are those which serve to stabilise the phosphate bond in the ribonucleotide molecules, or to stabilise the molecule as a whole or any part thereof. Examples of stabilising agents which may be used include sodium tripolyphosphate, orthophosphoric acid, and salts of ethylenediaminetetraacetic acid.

The invention may be used with conventional dog foods, such as that sold by the Applicants under the Trade Name "Purina Dog Chow". The flavour enhancers may be added to the dog food during its processing, or before or at the time that it is fed to the dog. For example, the compositions of the invention may be formulated by spraying the flavour enhancer, carried in an aqueous medium, on to the dog food during processing, prior to its packaging. On evaporation of the aqueous carrier, the flavour enhancer is carried in a dry form by the particles of dog food, and is distributed throughout the food. The flavour enhancer may also be added to the dog food in water used to moisten the food at the time of feeding. Yet again, the flavour enhancer may be mixed with a dry carrier and dusted on to the dog food.

The flavour enhancers used in the invention may be added to dry or moist dog foods of the various conventional types, and may also be used in combination with other additives, ingredients or food supplements.

The invention is illustrated by the following Examples.

Unless otherwise stated, the experiments described in the Examples used the dog food sold by the Applicants under the Trade Name "Purina Dog Chow". This product contains meat and bone meal, wheat germ meal, ground oat groats, ground yellow corn, ground grain sorghums, wheat middlings, ground wheat, soy bean meal, cereal food fines, dried whey, animal fat, vitamin B_{12} supplement, artificial colouring, pyridoxine hydrochloride, riboflavin supplement, brewer's dried yeast, vitamin F supplement, deactivated plant sterol, vitamin E supplement, thiamin, niacin, iodized salt, manganese sulphate, manganous oxide, zinc oxide, iron oxide, copper oxide and cobalt carbonate. By analysis, the product contains not less than 23% crude protein and 8% crude fat, and not more than 4.5% crude fibre and 10% ash.

The dogs used in the experiments included poodles, red-bone coonhounds, English setters, Labrador retrievers, springer spaniels, beagles, pointers and greyhounds. The dogs were randomly placed into groups each containing ten dogs, and there were five test dogs in each pen during the studies. The dogs were fed on an individual basis, the ration pans being rotated so that the same ration was not offered on the same side of the pen each day. The food consumption of each dog was calculated and recorded for the test period indicated in the results set out below.

The statistical significance of the test results was determined by using the Wilcoxon's matched-pairs signed-ranks test (American Statistical Association Journal, September 1965, pages 866—867).

In the tabulated results given in the tables below, the first figure under each ration column represents the total number of pounds of the ration which the group of dogs consumed, the dogs having equal access to both this ration and a second test or control ration, and the second figure (given in parentheses) represents the number of dogs which consumed more of the one ration in preference to the second ration. Where the second figure includes the fraction "½", this means that at least one dog showed equal preference for two rations.

EXAMPLE 1

The six materials set forth below were added in water to "Purina Dog Chow" at the time of feeding to determine whether the materials at the levels tested increase acceptability of the ration to the test dogs. The results are given in Table 1.

TABLE 1

Amount of Test Material (Fed Wet — Air-Dry Basis)	Ration Number A (Control) 0 ppm.	B 8 ppm.	C 80 ppm.	Significance
Test 1 (Guanine)				
Phase 1: 15 dogs — 5 days	31.6(3)	46.1(12)	—	P<.01
Phase 2: 15 dogs — 5 days	22.0(0)	—	523.1(15)	P<.01
Phase 3: 15 dogs — 5 days	—	36.6(6—1/2)	37.0(6—1/2)	N.S.
Test 2 (Guanosine)				
Phase 4: 15 dogs — 5 days	25.8(1—1/2)	47.3(13—1/2)	—	P<.01
Phase 5: 15 dogs — 5 days	30.2(2—1/2)	—	46.4(11—1/2)	P<.01
Phase 6: 15 dogs — 5 days	—	27.6(4)	43.1(11)	P<.01
Test 3 (Guanosine-3'-(2') phosphoric acid — sodium salt, hydrate)				
Phase 7: 15 dogs — 5 days	20.1(2)	41.7(13)	—	P<.01
Phase 8: 15 dogs — 5 days	22.0(2)	—	41.3(13)	P<.01
Phase 9: 15 dogs — 5 days	—	26.2(4)	38.8(11)	P<.01
Test 4 (Inosine)				
Phase 10: 15 dogs — 5 days	28.1(3)	42.2(12)	—	P<.03
Phase 11: 15 dogs — 5 days	25.6(4)	—	39.7(11)	P<.02
Phase 12: 15 dogs — 5 days	—	28.1(2—1/2)	41.3(12—1/2)	P<.01
Test 5 (Cytidine)				
Phase 13: 15 dogs — 5 days	32.1(4)	40.7(11)	—	N.S.
Phase 14: 15 dogs — 5 days	20.9(1/2)	—	49.2(14—1/2)	P<.01
Phase 15: 15 dogs — 5 days	—	30.3(6)	33.1(9)	N.S.
Test 6 (Uridine)				
Phase 16: 15 dogs — 5 days	27.2(4)	44.2(11)	—	P<.01
Phase 17: 15 dogs — 5 days	20.9(2)	—	44.8(13)	P<.01
Phase 18: 15 dogs — 5 days	—	23.7(4)	43.5(9)	N.S.

The results show that each of the six materials increased acceptability at both the 8 and 80 ppm. levels as compared with the control ration.

EXAMPLE 2

Example 1 was repeated with six additional materials as test materials using six pens of rotated dogs. The results are given in Table 2. The results show that the six materials effectively increased acceptability of the test rations compared with the control ration.

TABLE 2

Amount of Test Material (Fed Wet — Air-Dry Basis)	Ration Number A (Control 0 ppm.)	B 8 ppm.	C 80 ppm.	Significance
Test 1 (Guanine)				
Phase 1: 15 dogs — 5 days	34.6(2)	53.6(13)	—	P<.01
Phase 2: 15 dogs — 5 days	42.9(10)	—	42.3(5)	N.S.
Phase 3: 15 dogs — 5 days	—	38.2(5)	49.7(10)	P<.10
Test 2 (Guanosine)				
Phase 4: 15 dogs — 5 days	32.4(2—1/2)	57.0(12—1/2)	—	P<.001
Phase 5: 15 dogs — 5 days	33.3(2)	—	60.7(13)	P<.005
Phase 6: 15 dogs — 5 days	—	30.4(4)	51.0(11)	P<.02
Test 3 (Guanosine-3'-(2') phosphoric acid — sodium salt, hydrate)				
Phase 7: 15 dogs — 5 days	36.8(4)	50.3(11)	—	P<.04
Phase 8: 15 dogs — 5 days	31.3(3)	—	59.0(12)	P<.005
Phase 9: 15 dogs — 5 days	—	48.3(10)	45.1(5)	N.S.
Test 4 (Inosine)				
Phase 10: 15 dogs — 5 days	34.4(4)	55.3(11)	—	P<.03
Phase 11: 15 dogs — 5 days	33.0(1—1/2)	—	66.4(13—1/2)	P<.005
Phase 12: 15 dogs — 5 days	—	43.5(5)	52.5(10)	N.S.
Test 5 (Mixed isomers of 2'-cytidylic acid and 3'-cytidylic acid)				
Phase 13: 15 dogs — 5 days	35.2(4)	53.3(11)	—	P<.05
Phase 14: 15 dogs — 5 days	30.3(2)	—	63.9(13)	P<.005
Phase 15: 15 dogs — 5 days:	—	42.6(7—1/2)	51.0(7—1/2)	N.S.
Test 6 (2',3'-uridylic acid)				
Phase 16: 15 dogs — 5 days	31.9(3)	49.6(12)	—	P<.05
Phase 17: 15 dogs — 5 days	27.0(1—1/2)	—	59.5(13—1/2)	P<.001
Phase 18: 15 dogs — 5 days	—	44.5(4)	53.1(11)	N.S.

EXAMPLE 3

Examples 1 and 2 were repeated using guanosine alone and a mixture of guanosine and monosodium glutamate as the test materials. The guanosine used was obtained from two different sources of supply. The results are given in Table 3. The results show that the combination of guanosine and monosodium glutamate produced greater acceptability than guanosine alone.

TABLE

Ration Number			1	2	3	4	5
Ratio — "Purina Dog Chow" containing							
Guanosine, ppm.			0	4	8	0	4
Monosodium glutamate, ppm.			—	—	—	—	—
Feed Consumption — Fed Wet (Air-Dry Basis)							
Phase	No. Dogs	No. Days					
1	15	5	51.4(5)	59.6(10)	—	—	—
2	15	5	40.1(6—1/2)	—	45.8(8—1/2)	—	—
3	15	5	—	52.4(2—1/2)	67.1(12—1/2)	—	—
4	15	5	—	—	—	40.7(6)	44.4(9)
5	15	5	—	—	—	35.7(3—1/2)	—
6	15	5	—	—	—	—	32.6(2)
7	15	5	—	—	—	—	—
8	15	5	—	—	—	—	—
9	15	5	—	—	—	—	—
10	15	5	—	—	—	—	—
11	15	5	—	—	—	—	—
12	15	5	—	—	—	—	—

3

6	7	8	9	10	11	12	
8	0	4	8	0	4	8	
—	0	20	40	0	20	40	
							Significance
—	—	—	—	—	—	—	N.S.
—	—	—	—	—	—	—	N.S.
—	—	—	—	—	—	—	P<.01
—	—	—	—	—	—	—	N.S.
60.4(11—1/2)	—	—	—	—	—	—	P<.02
49.2(8)	—	—	—	—	—	—	N.S.
—	32.9(2)	58.3(13)	—	—	—	—	P<.001
—	37.7(6)	—	52.1(9)	—	—	—	N.S.
—	—	38.2(2)	60.0(13)	—	—	—	P<.005
—	—	—	—	22.5(3)	51.8(12)	—	P<.01
—	—	—	—	23.3(1)	—	56.6(14)	P<.001
—	—	—	—	—	37.3(7—1/2)	41.7(7—1/2)	N.S.

EXAMPLE 4

The effect of adding ribonucleic acid and hydrolyzed ribonucleic acid, respectively, in solution to "Purina Dog Chow" at the time of feeding to groups of test dogs was determined. The results are given in Table 4.

TABLE 4

Ration Number	1 (Control)	2	3	
% "Purina Dog Chow"	100	100	100	
RNA, ppm.	—	8	—	
Hydrolyzed RNA, ppm.	—	—	8	
Food Consumption Fed Wet (Air-dry basis)				Significance
Phase 1 30 dogs — 5 days	49.8(0)	103.7(30)		P<.001
Phase 2 30 dogs — 5 days	55.1(5—1/2)	—	89.0(24—1/2)	P<.001
Phase 3 30 dogs — 5 days	—	65.5(10)	87.0(20)	P<.03

The results show that both materials were effective in increasing acceptability.

EXAMPLE 5

The effect on the acceptability of dog rations of guanosine and a combination of guanylic acid, uridylic acid, cytidylic acid and adenylic acid added in solution to "Purina Dog Chow" at the time of feeding was determined. The results are set forth in Table 5.

TABLE 5

Ration Number	1 (Control)	2	3	
% "Purina Dog Chow"	100	100	100	
Guanosine	—	8	—	
2',3'-guanylic acid, ppm.	—	—	2	
2',2'-uridylic acid, ppm.	—	—	2	
2',3'-cytidylic acid, ppm.	—	—	2	
2',3'-adenylic acid, ppm.	—	—	2	
Food Consumption Fed Wet (air-dry basis)				Significance
Phase 1 15 dogs — 5 days	30.7(2)	50.9(13)	—	P<.005
Phase 2 15 dogs — 5 days	26.7(3)	—	61.1(12)	P<.005
Phase 3 15 dogs — 5 days	—	25.4(2)	50.3(13)	P<.001

The results show that the combination of the four named materials improved acceptability to a greater degree than guanosine alone.

EXAMPLE 6

The effect of various materials on the acceptability of dog rations was determined. The materials were added in water to the dog rations at the time of feeding. Each test group consisted of 10 dogs and the test period or phase in each instance was 5 days. The results are given in Table 6.

TABLE 6

	Food Consumption		
Test Material	Control ("Purina Dog Chow")	Test Material (10 ppm.) plus "Purina Dog Chow"	Significance
Purine	24.5(2—1/2)	42.2(7—1/2)	$P<.10$
Purine Riboside	22.4(0)	47.7(10)	$P<.005$
Pyrimidine	17.6(0)	29.4(10)	$P<.005$
Cytidine	15.3(0)	30.3(10)	$P<.005$
Thymidine	29.5(2)	40.1(8)	$P<.01$
Uridine	22.6(1)	47.1(9)	$P<.02$
Xanthosine	23.2(2)	39.1(8)	$P<.02$
2',-3'-Cyclic phosphate of Uridylic Acid	14.1(1)	33.1(9)	$P<.01$
Hypoxanthine	23.2(1—1/2)	41.2(8—1/2)	$P<.01$
Xanthine	27.5(2)	39.7(8)	$P<.10$
Uric Acid	10.7(0)	26.2(10)	$P<.005$
Cytosine	18.5(1)	28.9(9)	$P<.005$
5-Methyl Cytosine	19.4(1)	36.8(9)	$P<.01$
Uracil	18.2(1)	43.3(9)	$P<.01$

The results show that each of the materials tested improved acceptability of the ration when added at a level of 10 ppm.

EXAMPLE 7

The effect of brewer's dried yeast and hydrolyzed brewer's dried yeast on the acceptability of dog rations was determined. The materials were added in the water used to moisten the rations at the time of feeding. The results are given in Table 7. The "placebo" below was soy meal.

TABLE 7

Ration Number	1 (Control)	2	3	
"Purina Dog Chow" grams	452.67	452.67	452.67	
Control ("placebo"), grams	1.0	—	—	
Brewer's dried yeast, grams	—	1.0	—	
Hydrolyzed Brewer's dried yeast, grams	—	—	1.0	
Feed Consumption Fed Wet (air-dry basis)				Significance
Phase 1 30 dogs — 5 days	58.3	83.4(24)	—	P<.0001
Phase 2 30 dogs — 5 days	65.0(8)	—	89.8(22)	P<.005
Phase 3 30 dogs — 5 days	—	47.6(4—1/2)	107.0(25—1/2)	P<.0001

The results show that both materials increased acceptability of the action and that the hydrolyzed yeast material rendered the ration significantly more acceptable than did the non-hydrolyzed yeast material.

EXAMPLE 8

The effect of hydrolyzed brewer's yeast at two different levels on the acceptability of dog rations was determined. The yeast material was sprayed on "Purina Dog Chow" before feeding the test dogs. The results are given in Table 8.

TABLE 8

Ration Number	1	2	3	
% "Purina Dog Chow"	100	100	100	
Hydrolyzed brewer's yeast, grams/lb.	0	0.2	1.0	
Feed Consumption Fed Wet (air-dry basis)				Significance
Phase 1 30 dogs — 5 days	65.9(3)	116.5(27)	—	P<.0001
Phase 2 30 dogs — 5 days	—	75.3(3—1/2)	110.2(26—1/2)	P<.001
Phase 3 30 dogs — 5 days	62.0(4—1/2)	—	119.6(25—1/2)	P<.0001

The results show that the yeast material significantly improved acceptability of the dog ration both at 0.2 gms./lb. (440 ppm.) and at 1 gm./lb. (2,200 ppm.).

EXAMPLE 9

The effect of non-hydrolyzed and hydrolyzed brewer's yeast and torula yeast, respectively, on the acceptability of dog rations was determined. The materials in the form of aqueous media was sprayed onto the "Purina Dog Chow" ratio prior to feeding. The results are set forth in Table 9.

TABLE 9

Ration Number	1	2	3	4	5	6	
% Dog Chow	100	100	100	100	100	100	
% Control 1 (water)	—	—	—	—	—	—	
% Control 2 (water)	—	—	—	—	—	—	
% Non-hydrolyzed brewer's yeast	—	.25	—	—	—	—	
% Hydrolyzed brewer's yeast	—	—	.25	—	—	—	
% Non-hydrolyzed torula yeast	—	—	—	—	.25	—	
% Hydrolyzed torula yeast	—	—	—	—	—	.25	
Feed Consumption Fed Wet (air-dry basis)							Significance
Phase 1: 30 dogs — 5 days	27.45(5—1/2)	67.6(24.—1/2)	—	—	—	—	P<.0001
Phase 2: 30 dogs — 5 days	28.1(2)	—	85.7(28)	—	—	—	P<.0001
Phase 3: 30 dogs — 5 days	—	31.0(4)	71.1(26)	—	—	—	P<.0001
Phase 4: 30 dogs — 5 days	—	—	—	48.9(6—1/2)	77.6(23—1/2)	—	P<.01
Phase 5: 30 dogs — 5 days	—	—	—	52.0(8)	—	64.8(22)	P<.02
Phase 6: 30 dogs — 5 days	—	—	—	—	46.8(5)	71.7(25)	P<.0001

The results show that the two types of yeast, in both the non-hydrolyzed and hydrolyzed form, significantly improve acceptability and that the hydrolyzed yeast materials render the ration significantly more acceptable than do the non-hydrolyzed materials.

EXAMPLE 10

The effect of a period of three months storage on the acceptability of stabilized and unstabilized brewer's yeast materials was determined. The materials were sprayed onto the "Purina Dog Chow" rations prior to feeding. The results are given in Table 10.

TABLE 10

Ration Number	1	2	3	
% "Purina Dog Chow"	100	100	100	
Unstabilized hydrolyzed brewer's yeast, ml./lb. "Dog Chow"	7	—	—	
Hydrolyzed brewer's yeast stabilized with sodium tripolyphosphate and orthophosphoric acid, ml./lb. "Dog Chow"	—	7	—	
Hydrolyzed yeast stabilized with sodium salts of ethylene-diamine — tetraacetic acid and HCl, ml./lb. "Dog Chow"	—	—	7	
Feed Consumption Fed Wet (air-dry basis)				Significance
Phase 1 30 dogs — 5 days	53.9(3)	93.0(27)		$P<.0001$
Phase 2 30 dogs — 5 days	65.8(10)	—	84.6(10)	$P<.05$
Phase 3 30 dogs — 5 days	—	63.9(9)	87.7(21)	$P<.02$

The results show that the unstabilized material is inferior, as regards enhanced acceptability, to both of the stabilized materials.

EXAMPLE 11

The effect of hydrolyzation time and temperature on the acceptability of hydrolyzed brewer's yeast materials was determined. The various hydrolyzed materials were sprayed onto the "Purina Dog Chow" rations prior to feeding. The results are given in Table 11.

TABLE 11

Ration Number	1	2	3	4	5	6	
% "Purina Dog Chow"	100	100	100	100	100	100	
Brewer's yeast (1 gm./lb. "Dog Chow"), hydrolyzed at Temp. °C.	25	25	25	35	35	35	
No. hours processed	3	8	15	3	8	15	
Feed Consumption Fed Wet (air-dry basis)							Significance
Phase 1: 30 dogs — 5 days	90.3(11—1/2)	102.2(18—1/2)	—	—	—	—	N.S.
Phase 2: 30 dogs — 5 days	80.7(10)	—	104.5(20)	—	—	—	P<.04
Phase 3: 30 dogs — 5 days	—	70.1(5)	113.4(25)	—	—	—	P<.0001
Phase 4: 30 dogs — 5 days	—	—	—	59.2(10)	84.9(20)	—	P<.01
Phase 5: 30 dogs — 5 days	—	—	—	—	53.1(3)	101.4(27)	P<.0001
Phase 6: 30 dogs — 5 days	—	—	—	55.4(3—1/2)	—	103.9(26—1/2)	P<.0001
Phase 7: 20 dogs — 5 days	33.9(6—1/2)	—	—	62.1(13—1/2)	—	—	P<.01
Phase 8: 20 dogs — 5 days	—	46.0(7)	—	—	66.8(13)	—	P<.10
Phase 9: 20 dogs — 5 days	—	—	47.5(3—1/2)	—	—	64.3(16—1/2)	P<.005

The results show that products resulting from hydrolysis at 35°C. are more effective than those resulting from hydrolysis at 25°C. and that hydrolysis for fifteen hours at either temperature produces a more effective product that hydrolysis for shorter periods of time.

EXAMPLE 12

Commercial ribonucleic acid (10 g.) was added with mixing to 0.3N potassium hydroxide (200 ml.) until a clear light brown solution was obtained. This solution was incubated at 37°C. for a period of eighteen hours and then neutralized to a pH of about 8 with concentrated hydrochloric acid. The final volume was adjusted to 250 ml. with water. Using the same quantity of ribonucleic acid and neutralized potassium hydroxide as above (plus a small amount of additional potassium hydroxide to aid solution), a solution of non-hydrolyzed ribonucleic acid was prepared, as well as a blank containing neutralized potassium hydroxide only. These preparations were the preparations employed in the tests described in Example 4 with the results given in Table 4 being obtained.

EXAMPLE 13

Dry brewer's yeast (700 g.) was slurried with 0.3N sodium hydroxide (4000 ml.) in a Waring blender. The solution was incubated for a period of twenty-two hours at 37°C. and neutralized with concentrated hydrochloric acid. Water was added to bring the total volume to 5200 ml. A suspension of brewer's yeast (700 g.) in neutralized sodium hydroxide (4000 ml.) was prepared and diluted to 5200 ml. to serve as a control and a similar solution containing no yeast was prepared to serve as a blank. These materials were the materials employed in the tests described in Example 7 with the results given in Table 7 being obtained.

EXAMPLE 14

Each of three portions (600 g.) of dry brewer's yeast was slurried with water (2000 ml.). To this was added 9.2N potassium hydroxide (98 ml.) and water (900 ml.). After mixing, each suspension was incubated at 37°C. for eighteen hours and then neutralized to a pH of about 7 with concentrated hydrochloric acid. To one resulting hydrolyzate was added sodium tripolyphosphate (273 g.) plus enough phosphoric acid to bring the pH to about 7. To a second hydrolyzate was added disodium ethylenediamine-tetraacetate (41 g.). Nothing was added to the third hydrolyzate. Water was added to bring the volume of each preparation to 4200 ml. These preparations were the preparations employed in the tests described in Example 10 with the results given in Table 10 being obtained.

EXAMPLE 15

Dry brewer's yeast (770 g.) was slurried in water with the aid of a blender. To this was added 9.15N potassium hydroxide (130 ml.) and enough water to bring the total volume to 3900 ml. After eighteen hours incubation at 37°C., the resulting hydrolyzate was neutralized with concentrated hydrochloric acid (70 ml.), and diluted to 5b00 ml. One sample of the hydrolyzate (800 ml.) chloric acid (70 ml.), and diluted to 5400 ml.) and marked as supplement A. A second sample consisting of 4000 ml. of the hydrolyzate solution was marked as supplement B. A third sample consisting of 4000 ml. of water was marked as supplement C. These samples were the ones employed in the tests described in Example 8 with the results given in Table 8 being obtained.

EXAMPLE 16

Example 11 was repeated except that the hydrolysis conditions were varied as follows:

Ration No.	Conditions	
	Time	Temperature
1	16	37°C.
2	27	37°C.
3	40	37°C.
4	9	55°C.
5	16	55°C.
6	27	55°C.

The results of feeding the hydrolyzates produced to dogs showed that the products resulting from hydrolysis at the higher temperature and for longer periods of time tended to provide greater acceptability.

EXAMPLE 17

Dry brewer's yeast (17.5 lb.) was added to water (97.5 lb.), and the mixture slurried to an even consistency. To this was added potassium hydroxide (3.95 lb.) dissolved in water (4.0 lb.). This produced a concentration of 3.5 mcq. potassium hydroxide per gram of yeast. After a period of eighteen to fortytwo hours at room temperature, portions of the slurry were removed, neutralised with concentrated hydrochloric acid and sprayed onto "Purina Dog Chow". Upon being fed to dogs, it was found that rations containing the thus produced hydrolysate produced greater acceptability than did control rations.

WHAT WE CLAIM IS:—

1. A composition comprising a dog food and a flavour enhancer selected from the following: ribonucleosides, 2′,3′-ribonucleotides, poly-2′,3′-ribonucleotides, purine, pyrimidine, ribonucleic acid, chemically hydrolysed ribonucleic acid-containing materials, thymidine, xanthosine, hypoxanthine, xanthine, uric acid,

cytosine, 5-methyl cytosine, uracil, thymine, orotic acid, adenine, guanine, ureidosuccinic acid, dihydro-DL-orotic acid, dihydrouracil, dihydrothymine, dihydro-6-methyl uracil, allantoin and salts and mixtures thereof.

2. A composition according to claim 1, which contains at least four parts per million of said flavour enhancer, based upon the weight of the dog food.

3. A composition according to claim 2, which contains from 8 to 80 parts per million of said flavour enhancer, based upon the weight of the dog food.

4. A composition according to claim 1, which contains from 0.2 to 2.0 grams of said chemically hydrolysed ribonucleic acid-containing material per pound of dog food.

5. A composition according to claim 4, which contains approximately one gram of said chemically hydrolysed ribonucleic acid-containing material per pound of dog food.

6. A composition according to any preceding claim, which comprises a flavour enhancer produced by the hydrolysis of a ribonucleic acid-containing material in an aqueous medium, at a pH of at least 12, over a period of at least three hours.

7. A composition according to claim 6, in which said hydrolysis has been carried out at a temperature between 25°C and 55°C.

8. A composition according to claim 6 or claim 7, in which said hydrolysis has been carried out for a period between twelve and twentyfour hours.

9. A composition according to any claims 6 to 8, in which said hydrolysis has been carried out at a pH from 12.5 to 13.

10. A composition according to claim 9, in which said ribonucleic acid-containing material in yeast, and in which said hydrolysis has been carried out by the addition of approximately 3.5 milli-equivalents of an alkaline compound per gram of yeast.

11. A composition according to any preceding claim, which also comprises a salt of glutamic acid.

12. A composition according to claim 11, in which said salt is monosodium glutamate.

13. A composition according to any preceding claim, which also comprises a stabilising agent which is sodium tripolyphosphate, orthophosphoric acid, or a salt of ethylenediaminetetraacetic acid.

14. A composition according to claim 1, substantially as herein described in any of the foregoing Examples.

15. A process which comprises the addition to a dog food of a flavour enhancer selected from the following: ribonucleosides, 2',3'-ribonucleotides, poly - 2',3' - ribonucleotides, purine, pyrimidine, ribonucleic acid, chemically hydrolysed ribonucleic acid-containing materials, thymidine, xanthosine, hypoxanthine, xanthine, uric acid, cystosine, 5-methyl cytosine, uracil thymine, orotic acid, adenine, guanine, ureidosuccinic acid, dihydro-DL-orotic acid, dihydrouracil, dihydrothymine, dihydro-6-methyl uracil, allantoin and salts and mixtures thereof.

16. A process according to claim 15, in which there is added to said dog food at least four parts per million of the flavour enhancer, based upon the weight of the dog food.

17. A process according to claim 16, in which there is added to the dog food from 8 to 80 parts per million of the flavour enhancer, based upon the weight of the dog food.

18. A process according to claim 17, in which there is used from 0.2 to 2.0 grams of a chemically hydrolysed ribonucleic acid-containing material per pound of dog food.

19. A process according to claim 18, in which there is used approximately one gram of chemically hydrolysed ribonucleic acid-containing material per pound of dog food.

20. A process according to any of claims 15 to 19, in which said flavour enhancer is carried in an aqueous medium when added to the dog food.

21. A process according to claim 20, wherein the aqueous medium carrying said flavour enhancer is sprayed on to the dog food.

22. A process according to any of claims 15 to 21, wherein there is also added to the dog food a salt of glutamic acid.

23. A process according to any of claims 15 to 22, wherein there is also added to the dog food a stabilising agent which is sodium tripolyphosphate, orthophosphoric acid, or a salt of ethylenediamine-tetraacetic acid.

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